



TITLE:

# A MORPHOLOGICAL AND PHYSIOLOGICAL STUDY OF BONE SENSITIVITY

AUTHOR(S):

SUGIURA, YOSHINOBU

---

CITATION:

SUGIURA, YOSHINOBU. A MORPHOLOGICAL AND PHYSIOLOGICAL STUDY OF BONE SENSITIVITY. 日本外科宝函 1958, 27(3): 597-608

ISSUE DATE:

1958-05-01

URL:

<http://hdl.handle.net/2433/206639>

RIGHT:

# A MORPHOLOGICAL AND PHYSIOLOGICAL STUDY OF BONE SENSITIVITY

by

YOSHINOBU SUGIURA

From the 2nd Surgical Division, Kyoto University Medical School

(Director : Prof. Dr. YASUMASA AOYAGI)

Received for publication Feb. 20, 1958

## 1. INTRODUCTION

Surgeons have the experiences that fractures or osteomyelitis can evoke bone pain. However, the existence of sensory innervation is known only in the periosteum and reports on the sensitivity of the bone and bone-marrow are rare.

LERICHE (1930) detected pain in bone fracture occurring from the deep part of the bone but the author cannot find any report demonstrating this fact physiologically.

In his histological study of the nerves of the bone, de CASTRO (1925) used anesthetic agent in the decalcifying process of the bone-tissue and succeeded in impregnating nerves in bones. He reported that in developing bones the nerve fibers enter the ossifying layer and come into contact with the osteocytes, but they are not found in adult bones. He believed them all to be autonomic nerves.

MISKOLCZY (1926) described a special nerve termination ending in a fine nodule in the periosteum at the beginning of the Havers' canal.

O. SCHARTAU (1936) reported on the autonomic nervous innervation in the ossified vertebra of reptiles and D. J. HURRELL (1937) found some nerve fibers in the femur in the connective tissue between the bone cells and ending in those cells, and he postulated their role in the reflex arc to serve in the development and maintenance of bone cells. There have been many physiological studies of the autonomic nerves in the bone marrow, above all Morikawa, OKINAKA & ASAI demonstrated autonomic nerves in the bone marrow.

DUVERNEY reported that some nerve fibers enter the bone marrow running along the blood vessels through the nutritional canal.

W. GLASER (1928) described fine nervous networks along the arteries in the bone marrow.

TAKEYAMA found myelinated and non-myelinated nerve fibers running along or forming nerve plexuses in the adventitia of the nutritional arteries of the bone and only non-myelinated fibers in Havers' canal. He considered them to be sympathetic and parasympathetic fibers.

IKARI (1958) who studied the problem of pain sensitivity of the bone, reported some special sensory receptors in the periosteum and sensory nerve endings in the bone marrow; however he did not describe on their course.

The author tried to demonstrate the existence of sensory nerves in the bone tissues from the histological point of view, and also to establish their course.

## 2. MATERIALS AND METHODS

1) The materials used were from amputated femurs of adult human being and of adult dogs and cats.

These fresh bones were sawed into 1 or 2 cm thick specimens as soon as possible.

Stripping the periosteum from the bone marrow hasten the fixation and decalcification of bone specimens.

However, the author was careful to retain them as much as possible and to maintain the communities of nerve fibers in each specimen.

Guarding against breaking the bone marrow, the author some times removed the bone marrow tissue out of the bone and the isolated bone marrow was fixed in ceroidin and kept in 10% neutral formolalcohol solution until its staining.

2) The sawed specimens were kept in 10% neutral formol solution for fixation for over a week.

3) Decalcification.

The difficulty in the impregnation of bone nerves is in decalcification.

De CASTRO added chloral hydrate or urethane for the acceleration of the decalcification process. D. J. HURRELL used 96% alcohol for decalcification and further fixation of bone specimens.

According to the author's examination, the use of concentrated alcohol solution caused a marked shrinkage of the tissue and delayed the decalcification of the bone. Therefore, he used formol solution as the decalcifying solution according to SETO, and accelerated the decalcification process by warming the solution to 25° C as described by YOKOTA.

Composition of the decalcifying solution

60% pure nitric acid.....	6 cc
20 % formol solution.....	100 cc
Chloral hydrate.....	4 g

Other decalcifying solutions, for instance, 5% trichlor acetate which was added to formol at the rate of 10%, gave good results in the impregnation of bone nerves, but not so satisfying as the use of the solution above mentioned.

4) Following the decalcification the specimens were immersed in 5% potassium alum solution for 24 hours.

5) Taken from the potassium alum solution, the specimens were washed many times with fresh 10% formol solution.

6) Sliced with the freezing microtome into 30-40 $\mu$  frozen sections and kept in 10% neutral formol solution.

7) Impregnation of the axiscylinders was done by BIELSCHOWSKY's method modified by Seto or modified by SUZUKI after formalin fixation for over 6 months.

8) The myelin sheath was stained by EHRLICH's hematoxyline method after formalin fixation for several days.

## 3. MICROSCOPIC OBSERVATION

### 1) Periosteum

MISKOLCZY found in the periosteum of young mice winding nerve fibers, fine nervous networks, nerve endings with nodular expansions, and many VATER—PACINI corpuscles.

IKARI reported in the periosteum of human beings simple nonbifurcation free endings, terminal networks, complicated arborizing endings, and encapsulated glomerular corpuscles.

According to the author's study, the bones of human beings have abundant myelinated as well as non-myelinated nerve fibers which, remaining as nerve bundles or after repeated bifurcations, enter into the bones through HAVERS' canals mostly accompanying the vascular vessels.

As peripheral structure of the autonomic nerves in the periosteum, the terminal networks of SRÖHR or the nervous syncytia of JABONERO are impregnated. The author found throughout the periosteal layers thick nerve fibers which had myelin sheaths even in the far periphery (Fig. 3) and seemed to end freely (Fig. 1), or in arborizations (Fig. 4) near the contact surface with the bone.

In the layer of elastic fibers, there are glomerular corpuscles as special receptors, in which the nerves terminate in bifurcations or end-ear (Endöse).

The nodular nerve endings described by MISKOLCZY could not be found by the author.

### 2) The bone

The nerve fibers which enter the bone accompanying the blood vessels, run towards the bone marrow, but some branches seem to be distributed in the bone substance together with the blood vessels via HAVERS' canals opening on the bone surface.

The myelinated nerves in HAVERS' canals are more numerous in the metaphysis than in the diaphysis (Fig. 7).

Some myelinated fibers in HAVERS' canals run into the bone marrow and are distributed there accompanying the blood vessels in the bone marrow (Fig. 8).

In the axis cylinder staining the greater number of nerve fibers in HAVERS' canals have slightly winding courses (Fig. 9).

Some thick nerves are surrounded by coils of fine autonomic nerve fibrils (Fig. 10).

In the deeper portions of HAVERS' canals, some of the branches of the nerve bundles change into fine nerve fibrils while they run along the capillary blood vessels and coil around them (Fig. 11), but others take their course without any relation to the capillary blood vessels and are distributed on the endosteum of HAVERS' canals as fine fibrils (Fig. 12).

Terminal autonomic nerve networks were not found there.

In the endosteum of HAVERS' canals, especially thick nerve fibers terminated freely (Fig. 13), which, in the obliquely cut specimens, seems to enter the interstitial tissues between the osteocytes.

The author found a nerve fiber which took a winding course in a HAVERS' canal and ended in a loop (Fig. 14).

He could find neither nerve fibers which, as HURRELL described, course from the periosteum into the interstitial tissues of the osteocytes and run along HAVERS' canals, nor those which enter the interstitial tissues of the osteocytes from the HAVERS' canals and connect with osteocytes.

### 3) The Bone marrow

Concerning the nerve fibers in the bone marrow, IKARI reported the fundamental plexuses of BOEKE and, as sensory nerve endings, simple and plexiform terminations.

According to this author's study, the nerve bundles run along the vascular wall and form nerve plexuses here and there along their course, i. e. several thick nerve fibers are entangled and appear like large networks (Fig. 15, 16). Such nerve plexuses, as TAKEYAMA described, are well developed at the bifurcating portions of blood vessels.

The author also observed single or multiple nerve fibers which never form network structures, and fibers leaving the vascular wall, running in the bone marrow, with free endings (Fig. 17, 18); these were always simple without complicated terminal branches or special endapparati.

### 4) Degeneration Experiments

The author could find ganglion cells neither in the periosteum, nor in the bone tissue including the bone marrow.

Many workers of our laboratory histologically demonstrated the existence of afferent nerves in various viscera.

Since LANGLEY's description of autonomic nerves, it is well known that they have no interposing nerve cells in their course from the spinal cord to the tissues.

The afferent nerves also, including the visceral afferents, have no relay stations on the way and a single neuron, in the spinal ganglion, extends to the tissues.

Therefore, if, after posterior rhizotomy, we find degenerated nerves at the far peripheral portion in the viscera where the autonomic nerves have already changed their neurons, the degenerated nerves represent afferent nerves.

Many workers in our laboratory have demonstrated degenerated nerves after rhizotomy in the mucous membranes of the alimentary canals, urogenital organs, in the media of arteries and veins, and in the parenchymatous tissues in the liver, the pancreas, the ovary, the testis, and the mesenterial lymphnodes.

The author, supposing the bone and bone marrow may have similar nerve structures, conducted degeneration experiments on the afferent nerves.

Adult dogs were used. Laminectomy was done under intravenous sodium isomylal anaesthesia. The spinal cord was exposed, the posterior roots were cautiously separated from the anterior roots and the former were cut distal to the spinal ganglia.

Five days later a femur on the operated side was removed. The myelin sheaths were stained with EHRLICH's hematoxylin and the axis cylinders were stained by BIELSCHOWSKY's method modified by SUZUKI.

1) Posterior rhizotomy was done at  $L_1-L_4$ . Secondary degeneration of the nerves in the bone and the bone marrow was not found.

2) Posterior rhizotomy at  $L_1$ - $L_7$ . Marked nerve degeneration of the myelinated fiber bundles was observed in Havers' canals and in those nerves running along the arterial wall in the bone marrow (Fig. 19, 20).

3) Posterior rhizotomy at  $S_1$ - $S_3$

Secondary degeneration of the nerves in the femur was not found.

B. Physiological experiment on the sensitivity of the bone marrow.

TSUNEKAWA in our laboratory succeeded in demonstrating vascular sensitivity using a cardiac catheter with stimulating bipolar electrode at the end inserted into the bloodvessels.

Following his example the author made a hole in the distal end of a tibia and inserted the stimulating electrodes into the bone marrow as far as the central portion of the bone. Thus the bone marrow was electrically stimulated at a region where the periosteum and bone tissue were not injured.

1) Experimental method

Adult dogs were used. Anesthesia was induced with urethan and morphine.

A stimulus was given with the square wave (frequency, 60 c p&; duration, 5ms; intensity, 10-40 volt; concentric bipolar electrodes were set at one end of a nylon catheter 2 mm in diameter).

The nocireaction was recorded on a kimogram as a change in blood pressure and respiration.

2) Results

The kimogram showed only a slight and slow increase of blood pressure as the catheter was inserted into the bone marrow.

Electric stimulation of the bone marrow caused a marked increase of blood pressure.

For the sake of contrast, the author stimulated the same portion, after dissection of the sciatic and the femoral nerves at the foramen ischiadicum and at the inguinal ligament respectively; this resulted in no increase of blood pressure (Fig. 23).

The results metioned above verified the existence of sensitivity in the bone marrow.

## 5. DISCUSSION

Since the work of BOEKE & STOEHR, the peripheral structure of the autonomic nerve is considered to form a fine network called "Sympathische Grundplexus" by BOEKE and "Terminalreticulum" by STOEHR. They maintain that the nerve fibers never show free endings in the network.

SETO distinguished special nerve fibers in viscera which are much thicker than autonomic nerve fibers and terminate in free endings. He designated these nerves as visceral sensory nerves.

OTSU of our laboratory studied the visceral nerves which had been called sensory nerves by SETO and demonstrated that they are characteristic in having myelin sheaths even near the endings and show secondary degeneration in such peripheral tissues as the mucous membrane of the alimentary canals and the parenchymatous tissues in the ovary.

Orsu concluded from these results that they must be sensory in nature, because they fulfill the common character of sensory nerves in that they arise from the nerve cells in the spinal ganglia and single neurons reach the effector tissues.

The author found nerve fibers in the periosteum, bone and bone marrow which are thick and seem to end freely.

They are myelinated and show marked degeneration after posterior rhizotomy.

These facts confirm the existence of sensory nerves in the bone tissues.

These sensory nerves seem to terminate in free endings. However, TE LIN TSEI, who studied the afferent nerves in the liver, maintains that some visceral sensory nerves, though they apparently seem to terminate in free endings, sometimes change into fine fibrils forming networks like the autonomic nerves, terminal expansions or special end apparati. Therefore, the author considers that the free endings of the visceral sensory nerves must be more closely examined.

At any rate, the afferent nerves in the bone tissues can be distinguished by degeneration experiments in the peripheral portion where the autonomic nerves have already changed neurons.

The histological observations on the sensory nerves in bone were confirmed physiologically by means of electric stimulation of the bone marrow, which proved the existence of sensitivity by inducing nociceptions in animals.

The afferent nerves in the bone marrow, however, may exist not only for pain sensitivity, but may also play an important role in the physiological functions of the bone marrow, though these are not yet clarified.

## 6. CONCLUSION

Histological and physiological observations of the sensory innervation of bone lead us to the following conclusions:

- 1) Myelinated nerve fibers are more abundant in the periosteum than in the bone tissue and the bone marrow.

Some myelinated nerve fibers were found in the nerve bundles in Havers' canals, which enter the bone marrow.

- 2) In the periosteum, as IKARI described, the sensory endings are simple free endings, terminal arborizations and glomerular end-corpuscles.

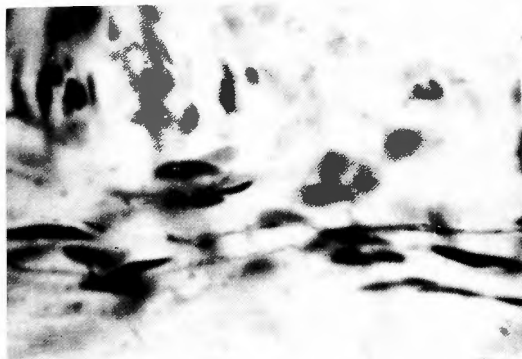
- 3) In HAVERS' canals of the bone, there are thick, winding sensory nerves and endings of terminal sensory apparatus.

- 4) The author could not find the nerves which D. J. HURRELL described as running through the interstitial tissues between osteocytes and having contact with osteocytes.

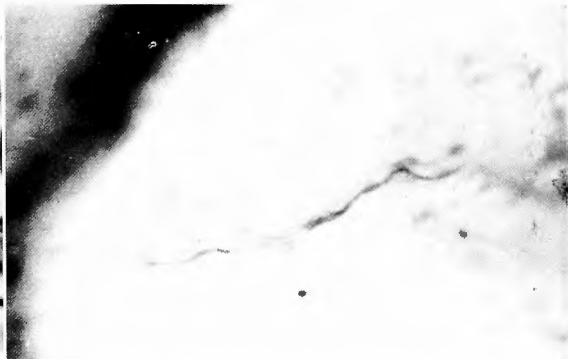
- 5) In the bone marrow, the author confirmed the existence of the simple free endings of the sensory nerves described by IKARI. However, they must be more closely examined before determining whether they are true or only apparent forms of terminal structures.

- 6) Posterior rhizotomy of the spinal cord caused secondary degeneration of the myelinated fibers in the bone tissue as well as in the bone marrow, proving





**Fig. 1.** Terminal network of the autonomic nerve in the periosteum, human being  $\times 900$ . Bielschowsky's method



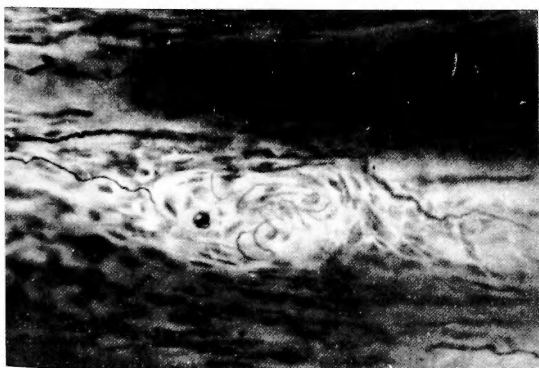
**Fig. 2.** Myelinated nerve fibers in the periosteum, Cat,  $\times 400$ . Ehrlich's hematoxylin method



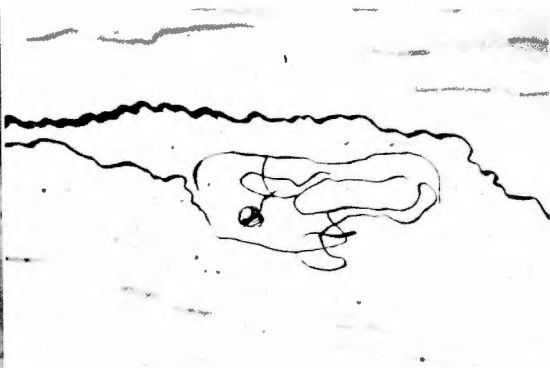
**Fig. 3.** Sensory nerve near its ending in the periosteum, human being,  $\times 400$ . Bielschowsky's method



**Fig. 4.** Arborized sensory ending in the periosteum, human being,  $\times 400$ . Bielschowsky's method

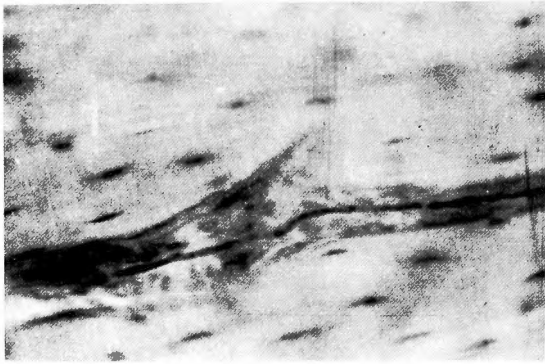


**Fig. 5.** A Glomerular end-apparatus of the sensory nerve in the periosteum, human being,  $\times 400$ . Bielschowsky's method

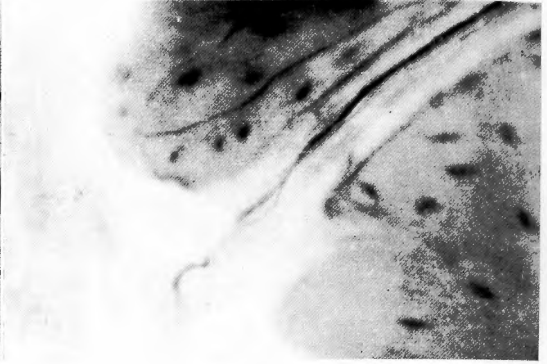


**Fig. 6.** Sketch of fig. 5

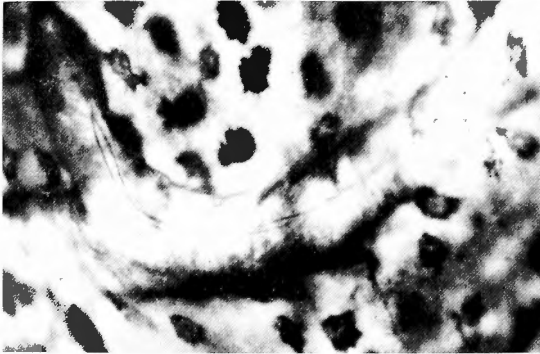




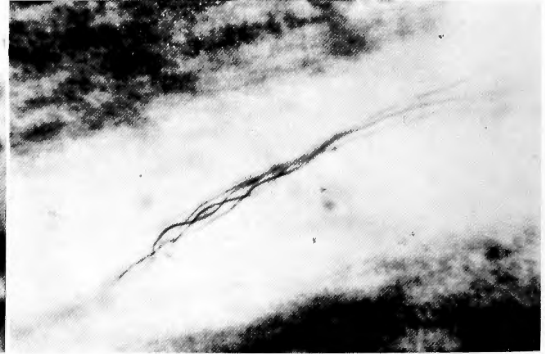
**Fig. 7** A normal myelinated nerve fiber running in Havers' canal in the diaphysis of the femur. Cat,  $\times 400$  Ehrlich's hematoxylin method



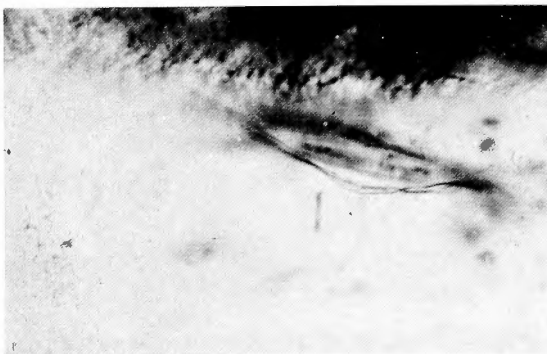
**Fig. 8** Normal myelinated nerve fibers in Havers' canal in the metaphysis of a femur. They are running to bone marrow. Cat,  $\times 400$ . Ehrlich's hematoxyline method



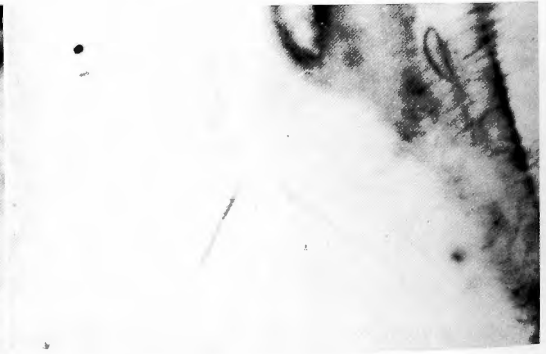
**Fig. 9** Two nerve fibers which run through Havers' canal not showing windings on the course. Cat,  $\times 400$  Bielschowsky's method



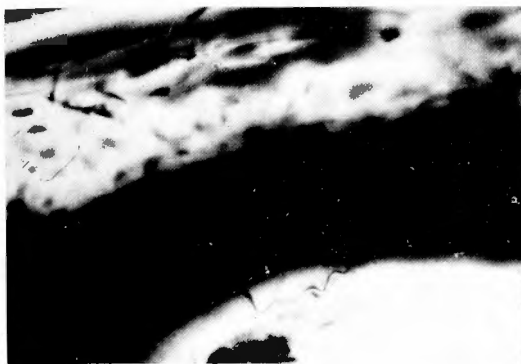
**Fig. 10** 10 Nerve fibers in Havers' canal showing a twist in their course. Cat,  $\times 400$ . Bielschowsky's method



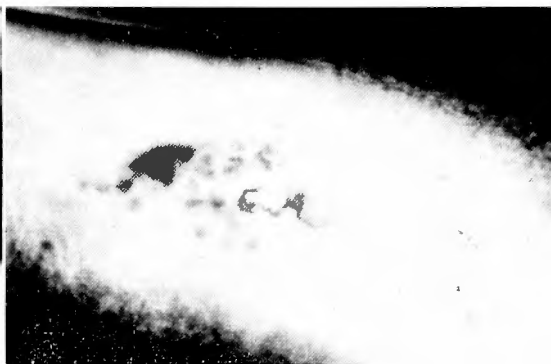
**Fig. 11** Fine nerve fibers (autonomic) found in Havers' canal. Cat,  $\times 900$ . Bielschowsky's method



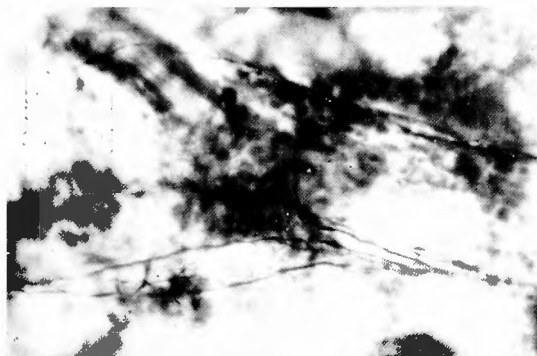
**Fig. 12** Fine nerve fibers in Havers' canal. Cat,  $\times 900$ . Bielschowsky's method



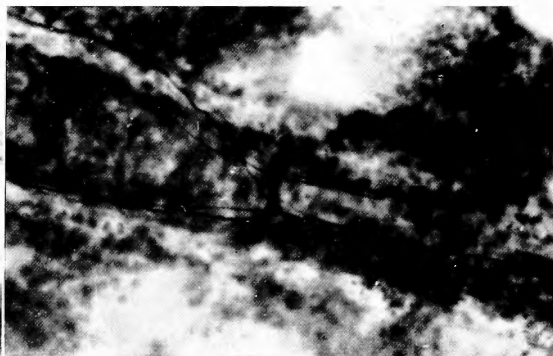
**Fig. 13.** A simple free ending of a sensory nerve in Havers' canal, human being,  $\times 400$ . Bielschowsky's method



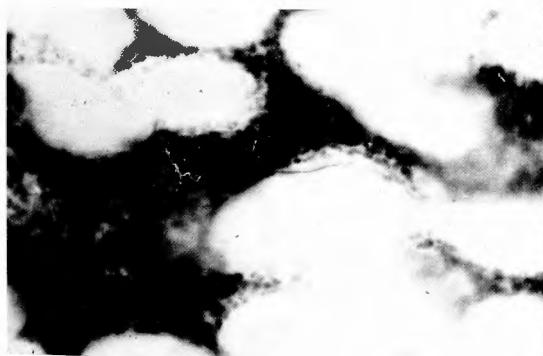
**Fig. 14.** A sensory ending forming a loop in its course in Havers' canal. Cat,  $\times 400$ . Bielschowsky's method



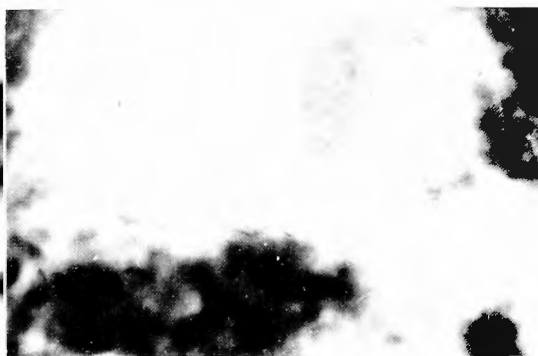
**Fig. 15.** A nerve plexus found on the wall of an artery in the bone marrow. Cat,  $\times 400$ . Bielschowsky's method



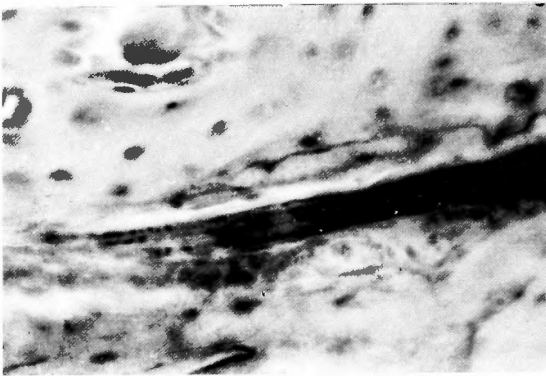
**Fig. 16.** A nerve plexus on the wall of an artery in the bone marrow. Cat.  $\times 400$ . Bielschowsky's method



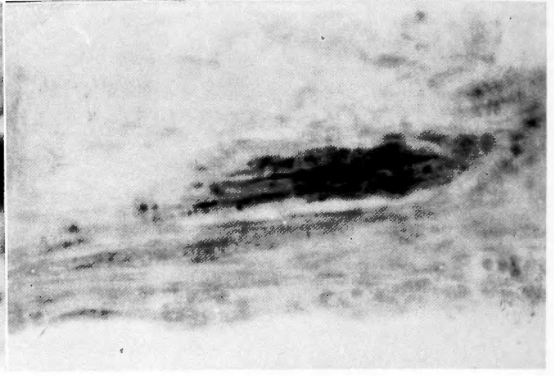
**Fig. 17.** A sensory free ending in the bone marrow tissue. Cat,  $\times 400$ . Bielschowsky's method.



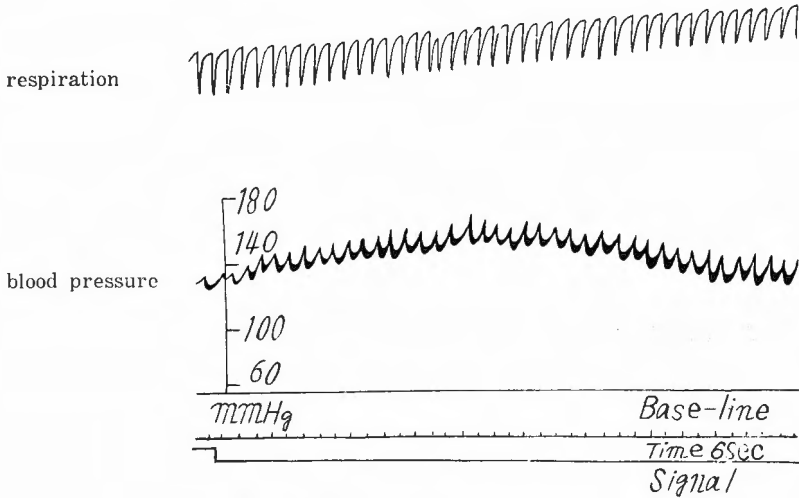
**Fig. 18.** High powered photograph of fig. 17  $\times 900$



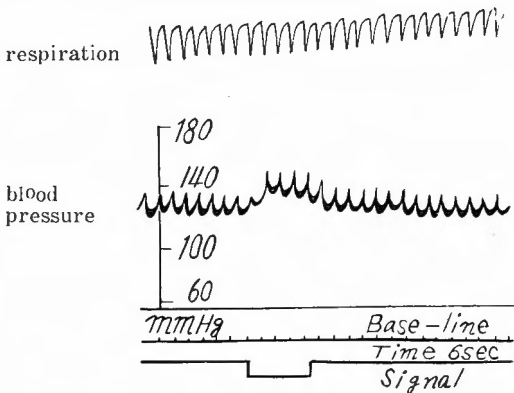
**Fig. 19.** Secondary degeneration of the myelin sheath of the nerves in Havers' canal following posterior rhizotomy on the lumbar segments. Dog,  $\times 400$  Ehrlich's hematoxyline method



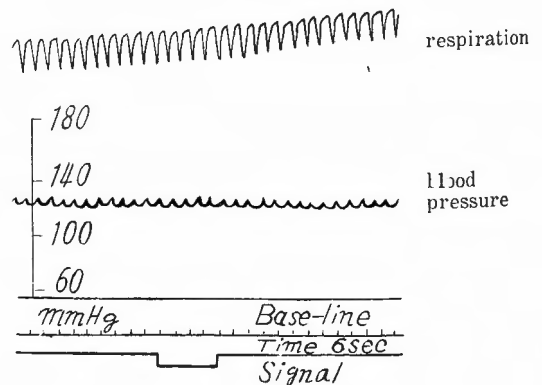
**Fig. 20** Degenerated myelinated nerves on the wall of an artery in the bone marrow following the same rhizotomy as in fig. 19. Dog'  $\times 400$  Ehrlich's hematoxyline method



**Fig. 21.** Nocireaction during the insertion of an electrode into the bone marrow. Respiration: above. Blood pressure: lower.



**Fig. 22.** Nocireaction during electric stimulation of the bone marrow of a femur. Dog



**Fig. 23.** Absence of the nocireaction during electric stimulation of the bone marrow of a femur after cutting the sciatic and the femoral nerves

the existence of sensory innervation of the bone and demonstrating the course of the nerves.

7) Electric stimulation of the bone marrow confirms the existence of sensitivity of the bone marrow.

8) The use of formol solution for the decalcification, and the fixation of specimens in formol solution for a long time after decalcification of the bone gave good results for the impregnation of the nerve fibers.

I am very grateful to our Assist. Prof. Ch. Kimura who gave me constant help and good suggestions throughout this work.

#### REFERENCES

- 1) Clark, W. E.: *The Tissues of the Body*. 3; 109, 1952.
- 2) de Castro, F.: *Technik zur Färbung des Nervensystems im Knochen*. Zbl. f. Allg. Path., **39**; 274, 1927.
- 3) Glaser, W.: *Bemerkungen Zur intramuralen Nerven-Versorgung der Blutgäse in knochenmark*. Z. Anat., **87**; 745, 1928.
- 4) Hurrell, D. J.: *The Nerve Supply of Bone*. J. Anat. **72**; 1937.
- 5) Ikari, T.: a) *The Histo-pathologic Study in Bone and Joint Tuberculosis*. (Kotsu kansetsu kekkaku no Byori-kaibo, in Japanese). J. Jap. Orth. Surg. Soc., **25**; 215, 1951. b) *On the Mecanism of Occuurence of Lumbago on its Neuro-Histological Research*. (Yôtsû Hassei Kiten ni Kansuru Kenkyu, in Japanese). J. Jap. Orth. Surg. Soc., **27**; 314, 1953. c) *Histological Study on Innervation of Human Bone*. Arch. Jap. Chir., **27**; 1, 1958.
- 6) Jabonero, J.: *Der Anatomische Aufbaudes Peripheren NeuroVegetativen Systems*. Acta. Neuro-Veg., Suppl. **4**, 1953.
- 7) Kimura, Ch.: a) *Development of the Dual Afferent Innervation Theory of the Viscera*. (Naizô-Chikaku Nijyu-Shihai Gakusetsu no Shinten, in Japanese). Nihon Rinshô, **11**; 85, 1953. b) *Visceral Sensory Nerves and Automic Nerves*. (Naizô-Chikaku Shinkeikei to Jiritsu Shinkeikei, in Japanese). Saishin Igaku, **9**; 34, 1954. c) *Peripheral Structure of Visceral Sensory Nerves*. (Naizô Chikaku Shinkei no Masshozo, in Japanese). Rinsho Geka, **9**; 25, 1954. d) *Physiology of Abdominal Pain*. (Fukutsu no Seiri, in Japanese). Rinsho no Shimpô, **7**; 1953.
- 8) Kimura, Ch., Otsu, A., Tanaka, N. and Inoue, H.: *A Systematic Histological Study of Sensory Endings in the Alimentary Canal*. Arch. Jap. Chir., **22**; 65, 1953.
- 9) Kure, T., and Okinaka, S.: *Autonomic Nerves System*. (Jiritsushinkeikei, in Japanese). 201, 1949.
- 10) Langley, J. N.: *The Autonomic Nervous System*. Cambridge, 1921.
- 11) Lilli, R. D.: *Decalcification of Bone in Relation to Staining*. Am. J. Clin. Path., **21**; 8, 1951.
- 12) Miskolczy, D.: *Über die Nervenendigungen der Knochenhaut*. Z. Anat. **81**; 638, 1926.
- 13) Powers, M. M.: *The Staining of hard Tissues with Silver*. Anat. Rec., **3**; 171, 1951.
- 14) Roshash, D. C.: *Decalcification of Bone with Trifluoroacetic Acid*. Am. J. Clin. Path., **22**; 914, 1952.
- 15) Schartau, C.: *Über die Innervation des in der Verknöcherung befindlichen Wirbers*. Z. Mikrok. Anat. Forch., **39**; 206, 1936.
- 16) Seto, H.: a) *Histological Studies on Visceral Sensation*. (Soshikigaku jo Kara mita Naizo no Chikaku, in Japanese). Igaku no Shinpo, **5**; 225, 1949. b) *Silver Impregnation for the Nervous System Being in Our Laboratory*. Tohoku. J. Exp. Med., **54**; 85, 1951.
- 17) Stöhr, Ph. Jr.: a) *Lehrbuch der Histologie und der Mikroskopische Anatomie des Menschen*. Springer Verlag, 1951. b) *Mikroskopische Anatomie des Vegetativen Nervensystems*. 1951. c) *Zusammenfassende Ergebnisse über die Endigungsweise des vegetativen Nervensystems*. Acta Neuro-Veg., **10**; 1-2, 1954.
- 18) Suzuki, K.: *Note of Technique to make Tissue-Preparations*. (Soshiki Hyôhon Seisaku Gijyutsu Nôto, in Japanese). (IV). Nôshinkei Ryôiki, **5**; 184, 1952.
- 19) Takeyama, K.: *Morphological Observation on the Distribution of Peripheral Nerves in the Bone-marrow*. (Kotsuzui ni Bunpu suru Massho-shinkei ni tsuite no Keitaigakuteki Kansatsu, in Japanese). Kyoto Pre. Med. J., **16**; 895, 1936.
- 20) Hiraki, K. & Tanaka, M.: *The Morphological Observation on the Distribution of Nerves in the Bone marrow*. (Kotsuzui nai no Shinkei Bunpu ni Kansuru Keitaigaku teki Kansatsu, in Japanese). Sogo Igaku, **12**; 727, 1955.

## 和 文 抄 録

## 骨の知覚に関する形態学的並びに生理学的研究

京都大学医学部外科学教室第2講座(指導 青柳安誠教授)

杉 浦 純 崑

BIELSCHOWSKY 氏神経鍍銀法の 瀬戸氏変法, 鈴木氏変法及び EHRlich 氏神経髄鞘染色法を応用して, 成人, 成犬, 成猫の大腿骨の標本に於て, 骨膜, 骨, 骨髓の知覚神経の形態及び分布を検索し, 更に脊髄後根を実験的に切断して, 骨, 骨髓内の末梢神経の二次的変性を追求し, 更に骨髓内に教室恒川の同心電極を挿入し電気刺激を行つて, 侵害反射を記録し, これ等の所見から骨の知覚に就いて, 次の結論を得た。

1) 骨膜, 骨, 骨髓に有髄神経が認められるが, その数的関係は, 骨膜に比べて骨, 骨髓には少ない。またハーベルス氏管から骨髓内に走る神経線維の中に, 有髄神経が存在することを認めえた。

2) 骨膜に, 猪狩の記述した遊離性の単純性知覚終末, 樹枝状分岐性終末並びに糸球状終末小体を認めた。

3) 骨のハーベルス氏管内に, 太い波状走行を示す知覚神経及び, 輪状知覚神経終末を認めた。

4) D. G. HURRELL の記述した, 骨細胞間質内を走り骨細胞に接する神経線維は認められなかつた。

5) 骨髓に於て, 猪狩の記述した実質内の単純性知覚神経終末を認めた。

6) 脊髄後根切断実験によつて, 骨及び骨髓に髄鞘変性像を認め, 知覚神経支配を受けていることが判明した。

7) 骨髓の電気刺激によつても, 骨髓が知覚支配を受けていることが判明した。

8) 骨の神経鍍銀には, 脱灰液にホルマリン水を使用し, 脱灰後ホルマリン水で長期間固定した方がよい結果を得られる。